

**EPA's Carson Beach & Wollaston Beach Bacterial Source Tracking Study:
Field Testing of a High-Through-Put Real-Time Quantificative PCR (HTP-RT-QPCR)
Assay for *Escherichia coli* & the *Enterococci* Fecal Indicator Bacteria***

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Current microbiological methods for determining water quality for recreational swimming and bathing at public and private beaches measure the viable concentrations of fecal indicator bacteria, *Escherichia coli* and *Enterococci* per 100-mL volume. These methods which utilize Membrane Filtration (Standard Method 1103.1, EPA Modified *E.coli* Method, EPA Method 1600 for *Enterococci*) and Idexx Quantitray (Colilert or Enterolert) require a 22- to 28-hr incubation period in addition to sample transport and processing times before certifiable counts of “viable” colony-forming-units (CFU) or most-probable-numbers (MPN) of *E. coli* or *Enterococci* counts can be obtained. Due to the episodic nature of pollution events (e.g., sewer surcharging, stormwater drainage, wild and domestic animal defecation, toilet flushing, boat bilge pumping, and the dilution of plumes by tidal, riverine, and spring flows, temporal change of water quality can be quite rapid. High-Through-Put (*htp*) DNA Isolation & Real-Time PCR Assays have been developed for quantifying the genomic DNA of *E. coli* and *Enterococcus* in natural surface waters as part of EPA's Carson Beach & Wollaston Beach Bacterial Source Tracking Study.

A highly *E. coli*-specific primer-hybridization-probe set and the IGS primer-hybridization-probe set from the Roche *Enterococcus M*-Grade Hybridization Probe Kit were used to optimize a DNA Isolation Protocol that utilized membrane filtration, the Roche MagNA Lyser (& “Green” Beads) and the Roche MagNA Pure LC Automated (*htp*) Platform. The optimized protocol was employed to purify genomic bacterial DNA from natural water samples collected at sites in North Dorchester Bay adjacent to Carson Beach (South Boston, MA) and sites along Wollaston Beach, Blacks Creek, and Furnace/Cunningham Brooks in Quincy, MA. Water samples were collected during “New Moon” tidal events at Carson Beach to detect potential leakage of Combined Sewer Overflows (CSOs) during the extreme low tides. Water samples were collected at the same sites along Carson Beach and at six Wollaston Beach stormwater drain outfalls and at sites in Black's Creek and along Furnace Brook during two storm events, one including significant snow melt. Concentrations of PCR-quantifiable genomic equivalents (GEQs) of *E. coli* and *Enterococcus* (*E. faecalis* & *E. faecium*) were compared with concentrations of “viable” bacteria quantified using respective membrane filtration and Quantitray assays.

Interesting trends were observed in the concentrations of GEQs, CFUs, and MPNs as well as the GEQ/CFU and GEQ/MPN ratios along Furnace Brook as it flowed into Black's Creek Salt Marsh area and emptied into the southern end of Quincy Bay. Extremely high numbers of *Enterococci* (GEQs, CFU, & MPN) were observed in stormwater outfall and gutter flows from Wollaston Beach. Contrary to expectations, the seepage interfaces along Wollaston Beach contained significant numbers of fecal bacteria. Samples collected at some sites along Carson Beach during extreme low tides had high numbers of fecal bacteria suggesting discharges from CSO outfall pipes. CSO-087 at the southern end of Carson Beach, whose outfall pipe is broken, has continuous upwelling flows through the sand under all conditions and phases of the tidal cycle. Its fresh water flows, which tend to hug the shoreline, appear to be a significant source of fecal loading to North Dorchester Bay. It may be the source of fecal indicator bacteria exceedances that result in "No Swimming" postings by the Massachusetts Department of Urban Parks & Recreation (DUPR formerly the MDC), especially on days during the summer that follow extremely low tides.

In summary, the HTP-RT-QPCR Assays developed for isolation and quantification of *E. coli* and *Enterococcus* DNA may serve as an important tool in testing recreational and drinking waters for fecal indicator bacteria as well as Total Maximum Daily Loading (TMDL) determinations for natural water bodies. The second part of the Carson Beach Bacterial Source Tracking Study, which involves development of a "host-specific" primer-probe set for differentiating mammalian host of fecal indicator bacteria in a HTP-RT-QPCR Assay, is underway.

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